Biomarkers for Alzheimer s disease

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2015

document version
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CHAPTER 6

Increase in CSF F_2-isoprostanes is related to cognitive decline in APOE4 carriers

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J. Alzheimers Dis. 2013;36(3):563-70.

ABSTRACT

In this longitudinal study we investigated the effect of Apolipoprotein E (APOE) genotype on the relation between cognitive decline and cerebrospinal fluid (CSF) F_2-isoprostanes, the reference marker for oxidative stress. Twenty non-demented subjects, 58 mild cognitive impairment (MCI) patients and 63 Alzheimer disease (AD) patients with measurements of CSF F_2-isoprostanes at two time points (with a mean interval of 2.0 ± 1.1 years) and known APOE genotype were included. Mean clinical follow-up time was 3.9 ± 2.4 years. For change in F_2-isoprostanes over time and associations with MMSE age- and sex-adjusted linear mixed models were used. Analyses were done for APOE4 carriers and non-carriers separately. In APOE4 carriers annual change in F_2-isoprostane levels appeared larger than in APOE4 non-carriers (β[SE] 2.5[0.5], $p < 0.001$ vs. 1.8[0.5], $p < 0.01$). In addition, increase in F_2-isoprostanes was associated with further cognitive decline in APOE4 carriers ($p < 0.05$), but not in non-carriers ($p = 0.28$). Our results reiterate the importance of oxidative stress in neurodegeneration, especially in APOE4 carrying patients. Future studies should focus on the possibility of increased vulnerability to oxidative damage in APOE4 carriers.

1. Introduction

The Apolipoprotein ε4 allele (APOE ε4) is an important risk factor for Alzheimer's disease (AD): carriers of this allele develop AD at a higher annual rate.[1] The APOE4 allele is associated with lower levels of cerebrospinal fluid (CSF) Amyloid-β 1-42 ($A\beta_{42}$), and higher $A\beta_{42}$ deposition as measured with positron emission tomography (PET) even in cognitively healthy subjects.[2-6] However, APOE4 carriership has also been associated with cardiovascular and cerebrovascular disease and worse outcome after traumatic brain injury.[7-9] The mechanism through which APOE exerts these effects is still not completely known. Processes such as neuroinflammation and impaired CNS repair mechanisms have been implicated in APOE4 carriers.[9-12] This suggests that additional, more non-specific pathways contribute to the risk of AD in APOE4 carriers.

F_2-isoprostanes are a sensitive marker for lipid peroxidation due to oxidative stress.[13] There are numerous isomers of F_2-isoprostanes, but iPF2α-VI is the one most extensively studied in Alzheimer's disease. There is increasing evidence that F_2-isoprostanes in brain and CSF are increased in patients with AD compared to controls.[14-20] Several studies have reported increased accuracy in differentiating AD from controls, mild cognitive impairment (MCI) and other dementias when F_2-isoprostanes were added to other CSF or MRI measures.[19, 21, 22] Longitudinally, CSF F_2-isoprostanes appear to increase over time and correlate with disease duration and severity of symptoms in MCI and AD.[18, 21-24] Recently, we showed that non-specific CSF markers changed over time while AD-specific markers did not, and of all non-specific markers tested, F_2-isoprostane concentration showed the largest effect over time.[24]

Only a few small studies examined the relation between APOE genotype and F_2-isoprostanes. In a cross-sectional study in cognitively healthy individuals the APOE4 carriers had a higher CSF F_2-isoprostane concentration compared to non-carriers,[25] providing support for the notion that oxidative stress plays a role in APOE4 carriers. In the present study we investigated the influence of APOE4 on the change in F_2-isoprostanes over time as well as on the relation between F_2-isoprostanes and rate of cognitive decline in a longitudinal cohort of patients from our memory clinic.

2. Materials and methods

2.1 Patients

From a prospective longitudinal cohort of our memory clinic,[24] 141 patients with known APOE genotype were included. There were 20 non-demented subjects, 58 MCI and 63 AD patients. All patients underwent standard dementia screening at baseline, including physical and neurological examination, EEG, MRI and laboratory tests. Cognitive screening included at least a Mini Mental State Examination (MMSE) but more often a comprehensive

neuropsychological test battery. The diagnoses of AD and MCI were made according to standard criteria (NINCDS-ADRDA,[26] and Petersen[27]) by consensus in a multidisciplinary team without knowledge of the CSF results. The non-demented group consisted of 16 patients with subjective memory complaints, 2 patients with a psychiatric disorder and 2 patients with temporal epilepsy. The diagnosis of subjective memory complaints was made when the results of all clinical examinations were normal. All subjects gave written informed consent and the study was approved by the local ethical review board.

2.2 Follow-up

Patients underwent a second lumbar puncture with a minimum interval of 8 months, and mean±SD of 2.0±1.1 years. Most patients were followed clinically for a longer period, with a mean±SD duration of 3.9±2.4 years. Within the MCI group 15 patients remained stable (sMCI), 33 progressed to AD, 3 to frontotemporal lobar degeneration (FTLD), 3 to vascular dementia (VaD), 1 to dementia with Lewy bodies (DLB), 1 to progressive supranuclear palsy (PSP), 1 was diagnosed with temporal epilepsy and 1 was diagnosed with normal pressure hydrocephalus. From the 20 non-demented subjects 10 patients remained stable, while 6 patients with subjective memory complaints progressed to MCI, 3 to AD, and 1 to VaD. We used MMSE to estimate cognitive decline over time. There were 669 MMSE scores of 121 patients available (median 4, range 2-17).

2.3 CSF biochemical analyses

CSF was obtained by lumbar puncture, using a 25-gauge needle, and collected in 10 mL polypropylene tubes (Sarstedt, Nümbrecht, Germany). Within two hours, CSF samples were centrifuged at 1800*g* for 10 minutes at 4° C. CSF was aliquoted in polypropylene tubes of 0.5 or 1 mL (Sarstedt) and stored at -80° C. Baseline $A\beta_{42}$, tau and ptau-181 were measured with commercially available ELISAs (Innotest β-amyloid$_{(1-42)}$, Innotest hTAU-Ag and Innotest Phosphotau$_{(181P)}$; Innogenetics, Ghent, Belgium) on a routine basis as described before.[28] The performance of the assays was monitored with pools of surplus CSF specimens, one in the normal and one in the AD range. In the study period multiple specimens were included in over 18 runs for this purpose. The inter-assay coefficients of variation (CV) (mean±SD) were 11.3±4.9 % for $A\beta_{42}$, 9.3±1.5 % for tau and 9.4±2.5 % for ptau-181.

The concentration of iPF2α-VI (F_2-isoprostanes) at baseline and follow-up was determined by liquid chromatography tandem mass spectrometry (LC-MS/MS), as described before.[24] Baseline and follow-up samples were analysed in the same assay to prevent inter-assay variability. Intra-run CV was 4.8%, and inter-run CV 7.6% at 199 pg/mL and 10.1% at 43 pg/mL (n = 17). The team involved in the CSF analysis was not aware of the clinical diagnoses.

2.4 APOE genotyping

For APOE genotyping, DNA was isolated from 10 mL EDTA blood by the QIAamp DNA blood isolation kit from Qiagen. The genotype was determined with the Light Cycler APOE mutation detection kit (Roche Diagnostics GmbH, Mannheim, Germany). Subjects were classified as APOE4 carriers if they had one or two ε4 alleles, and as non-carriers if they had no ε4 alleles.

2.5 Statistical analysis

For statistical analysis, SPSS version 15.0 (Windows) was used. Frequency distributions for categorical variables were compared with chi-squared tests. For assessing differences between groups ANOVA with post-hoc Bonferroni corrections or Student's T-tests were used when appropriate. We performed linear mixed models to estimate annual change in MMSE over time per diagnostic group, stratified by APOE4 carriership. Time (continuous variable), diagnosis (categorical variable) and interaction of time and diagnosis were entered as independent variables, while MMSE was the dependent variable. Furthermore, linear mixed models were used to investigate the effect of APOE4 on longitudinal change in F_2-isoprostane concentration and on the relation between F_2-isoprostanes and cognitive decline. This analysis accounts for within-subject correlations over time, is suitable for varying time intervals between assessments, and allows different numbers of assessments per subject. Therefore this method has increased statistical power, as all assessments can be used in the analysis. All analyses were stratified for APOE4 carriership to estimate the effects for APOE4 carriers and non-carriers separately. First, we used linear mixed models to estimate change in F_2-isoprostanes over time. Time (continuous variable) was entered as independent variable, while F_2-isoprostane concentration was the dependent variable and we adjusted for age, sex and diagnosis (categorical variable). A random intercept and slope with time were assumed. Subsequently, we used linear mixed models to assess the association between annual change in F_2-isoprostane concentration and rate of cognitive decline as defined by repeated MMSE. Annual change in CSF F_2-isoprostanes was calculated as follows: value at follow-up minus value at baseline, divided by duration of follow-up in years. Annual change in F_2-isoprostanes, time, and interaction between time and annual change in F_2-isoprostanes were independent variables, while MMSE score was the dependent variable. We adjusted this analysis for age, sex and diagnosis (categorical variable). All MMSE scores were taken into account and a random intercept and slope with time were assumed. For illustrative purposes we used Pearson's correlations with annual change in F_2-isoprostanes and annual change in MMSE (calculated as follows: value at follow-up minus value at baseline, divided by duration of follow-up in years). Overall, the level of significance was set at $p < 0.05$.

3. Results

Table 1 shows the patient characteristics according to APOE4 status and diagnosis. There were 85 APOE4 carriers and 56 non-carriers. There were no differences in age and follow-up time between APOE groups or diagnostic groups. There was a non-significant trend for difference in sex between APOE4 carriers and non-carriers (p=0.08). Within the APOE4 carriers there were more AD patients compared to the non-carriers. Within the non-demented and MCI subgroups the APOE4 carriers had lower MMSE at follow-up than non-carriers. Values of CSF $A\beta_{42}$ were lower, while tau and ptau-181 were higher in APOE4 carriers compared to non-carriers within non-demented and MCI subgroups. There were no differences in MMSE or CSF biomarker concentrations between APOE4 carriers and non-carriers within the AD subgroup.

We used age, sex and diagnosis adjusted linear mixed models to investigate the effect of time on CSF F_2-isoprostane levels. In both APOE4 carriers and APOE4 non-carriers, there was no main effect of diagnosis (p=0.20 and p=0.63), indicating similar CSF F_2-isoprostane concentrations in all diagnostic groups. In APOE4 carriers CSF F_2-isoprostane concentration increased 2.5±0.5 pg/mL per year. In APOE4 non-carriers this increase appeared to be smaller, i.e. only 1.8±0.5 pg/mL per year (table 2). When we repeated the analyses after exclusion of MCI patients converting to another type of dementia (*n*=9) and non-demented subjects progressing to dementia (*n*=4), results remained essentially unchanged (APOE4 carriers β±SE 2.6±0.6, p<0.001 and non-carriers β±SE 1.8±0.5, p=0.001). When the analysis was repeated after exclusion of two outliers with extreme increase in F_2-isoprostane levels at follow-up, results were essentially unchanged.

Table 1. Patient characteristics for the separate APOE4 groups per diagnostic category

	APOE4 non-carriers				**APOE4 carriers**			
	Total (*n* = 56)	*Non-demented (n = 12)*	*MCI (n = 24)*	*AD (n = 20)*	Total (*n* = 85)	*Non-demented (n = 8)*	*MCI (n = 34)*	*AD (n = 43)* [§)]
Age (years) mean ± SD	65 ± 9	63 ± 11	67 ± 9	65 ± 8	67 ± 8	68 ± 9	69 ± 8	65 ± 8
Sex, *n* (%) female	16 (29%)	2 (17%)	8 (33%)	6 (30%)	38 (45%)	3 (38%)	13 (38%)	22 (51%)
MMSE baseline	26 ± 3	28 ± 1	27 ± 2	23 ± 4 [a, b]	24 ± 5 [c]	28 ± 2	26 ± 3	22 ± 6 [a, b]
MMSE follow-up *	22 ± 8	28 ± 1	25 ± 4	15 ± 7 [a, b]	18 ± 7 [c]	24 ± 4 [d]	19 ± 5 [d]	17 ± 7 [a]
Annual change in MMSE **	-1.3(0.2)	-0.1(0.5)	-0.7(0.3)	-2.4(0.3) [a, b]	-1.8(0.2)	-0.7(0.6)	-1.6(0.3)	-1.9(0.3) [a]
Clinical follow-up (years)	3.5 ± 2.2	3.9 ± 3.1	3.4 ± 2.3	3.5 ± 1.6	4.1 ± 2.5	6.0 ± 3.4	4.3 ± 2.3	3.5 ± 2.4
Time between LP's (years)	2.0 ± 1.1	2.3 ± 1.7	2.1 ± 1.1	1.7 ± 0.7	2.0 ± 1.1	2.5 ± 1.8	2.0 ± 1.1	1.9 ± 1.0
$A\beta_{42}$ (pg/mL)	614 ± 284	782 ± 274	671 ± 306	444 ± 158 [a, b]	437 ± 162 [c]	632 ± 289	435 ± 133 [a, d]	403 ± 125 [a]
tau (pg/mL)	482 ± 326	280 ± 99	393 ± 253	710 ± 368 [a, b]	686 ± 461 [c]	503 ± 276 [d]	744 ± 584 [d]	675 ± 368
ptau-181 (pg/mL)	67 ± 37	45 ± 16	57 ± 31	92 ± 39 [a, b]	86 ± 39 [c]	81 ± 32 [d]	90 ± 45 [d]	85 ± 35
CSF F_2-isoprostane concentration (pg/mL)								
Baseline	14.5 ± 4.3	15.0 ± 4.3	14.3 ± 4.7	14.4 ± 4.0	15.7 ± 4.7	16.4 ± 5.3	16.5 ± 4.8	14.8 ± 4.4
Follow-up	18.0 ± 7.5	15.9 ± 3.8	19.9 ± 7.5	17.0 ± 8.9	20.3 ± 9.0	25.9 ± 13.6	20.8 ± 9.5	18.8 ± 7.2
Annual change F_2-isoprostane ***	2.1 ± 5.4	0.8 ± 2.0	3.3 ± 3.9	1.5 ± 7.8	3.1 ± 5.8	6.9 ± 11.4	2.6 ± 5.1	2.7 ± 4.6

* Given values are the last available MMSEs (available for 121 patients)

** Calculated with linear mixed models, to make use of all available MMSE values. Time (continuous variable), diagnosis (categorical variable) and interaction between time and diagnosis were entered as independent variables, while MMSE was the dependent variable. The analysis was stratified according to APOE status. Given values are main effects of time, not adjusted for age and sex.

*** Calculated as follows: value at follow-up minus value at baseline, divided by duration of follow-up in years.

[a] $p < 0.05$ vs non-demented subjects from the same APOE group, [b] $p < 0.05$ vs MCI subjects from the same APOE group

[c] $p < 0.05$ vs APOE4 non-carriers (total APOE groups), [d] $p < 0.05$ vs APOE4 non-carriers (in patients from the same diagnostic group)

Table 2. Results of the mixed models analyses per APOE group

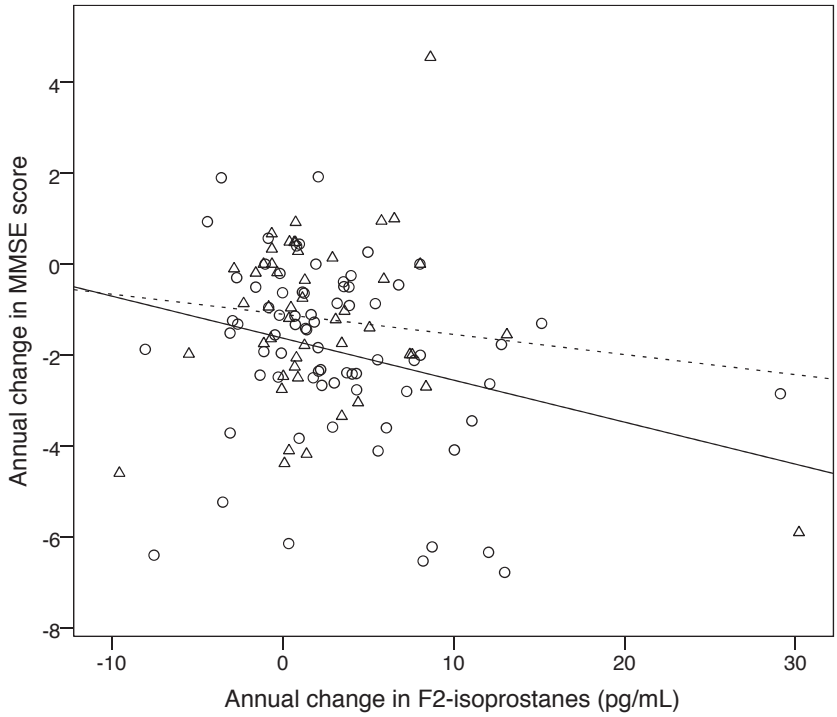
	APOE4 non-carriers	APOE4 carriers
1st analysis [a]		
Change of F_2-isoprostanes over time	1.8 (0.5), p < 0.01	2.5 (0.5), p < 0.001
2nd analysis [b]		
Change of MMSE over time	-1.1 (0.3), p < 0.001	-1.4 (0.2), p < 0.001
Association change of F_2-isoprostanes with baseline MMSE	-0.01 (0.06), p = 0.81	0.05 (0.09), p = 0.53
Association change of F_2-isoprostanes with change of MMSE	-0.05 (0.05), p = 0.28	-0.09 (0.04), p = 0.02

[a] Data are presented as β(SE), p-value. Age-, sex- and diagnosis-adjusted linear mixed models were used with time (as continuous variable) as independent variable and CSF F_2-isoprostane level as dependent variable. A random intercept and a random slope with time were assumed. The main effects of diagnosis are not shown as none of these effects was significant (crude values are displayed in Table 1). The main effect of time represents annual increase of F_2-isoprostane levels for each APOE group separately.

[b] Data are presented as β(SE), p-value. Age-, sex- and diagnosis-adjusted linear mixed models were used with annual change in F_2-isoprostanes, time, and interaction between time and annual change in F_2-isoprostanes as independent variables, while MMSE was the dependent variable. A random intercept and a random slope with time were assumed. The main effect of time represents the change of MMSE over time, adjusted for age, sex and diagnosis (for unadjusted annual change in MMSE per APOE group and diagnostic subgroup we performed a separate mixed model analysis, results are shown in Table 1). The main effect of annual change in F_2-isoprostanes represents the association between annual change in F_2-isoprostanes and baseline MMSE, while the interaction with time represents the association with change in MMSE. As the clinical follow-up was almost twice as long as the time between the two lumbar punctures, the interaction in the ε4 carriers suggests that annual change in CSF F_2-isoprostanes was related to further cognitive decline.

Subsequently, we used age, sex and diagnosis adjusted linear mixed models to assess the associations between annual change in F_2-isoprostane concentration (average time between LP's 2.0±1.1 years) and subsequent cognitive decline as measured using MMSE (average clinical follow-up 3.9±2.4 years). There were no associations between annual change in F_2-isoprostanes and baseline MMSE in neither APOE4 carriers nor non-carriers (p=0.53 and p=0.81 respectively; table 2). There was a main effect of time however: in APOE4 carriers MMSE decreased on average with 1.4 points per year, and in APOE4 non-carriers MMSE decreased by 1.1 point per year (both p<0.001). For APOE4 carriers we found an association between change of MMSE and annual F_2-isoprostane change, while in APOE4 non-carriers there was no association. This suggests that change in F_2-isoprostanes predicts the rate of further cognitive decline in APOE4 carriers, but not in non-carriers. When we repeated the analysis after exclusion of two outliers with extreme increase of F_2-isoprostanes over time, results remained essentially unchanged. Figure 1 provides a scatter plot of the association between annual change in CSF F_2-isoprostanes and annual change in MMSE, for APOE4 carriers (Pearson's r: -0.27; p=0.02) and non-carriers (Pearson's r: -0.14; p=0.35) separately.

Figure 1. Association of annual change in F_2-isoprostane levels and MMSE over time.



Linear mixed models were used with annual change in F_2-isoprostane level, time, diagnosis and interaction between time and annual change in F_2-isoprostane level as independent variables, and MMSE score as dependent variable. For illustrative purposes we used Pearson's correlations with annual change in F_2-isoprostane level and annual change in MMSE score (calculated as follows: value at follow-up minus value at baseline, divided by duration of follow-up in years). The solid line and dots represent the APOE4 carriers (Pearson's r: -0.27; p = 0.02), the dashed line and triangles the non-carriers (Pearson's r: -0.14; p = 0.35).

4. Discussion

In this longitudinal study we found a larger increase over time in the CSF level of F_2-isoprostanes in APOE4 carriers than in APOE4 non-carriers. Moreover, annual change in CSF F_2-isoprostanes was related to further cognitive decline measured by repeated MMSE in APOE4 carriers, but not in non-carriers. These results support the theory that oxidative stress is more important in APOE4 carriers than in non-carriers in the neuropathological cascade leading to cognitive decline in the context of AD.

Apolipoprotein E (ApoE) is a lipoprotein involved in cholesterol and lipid transport. It is abundant in brain and CSF, where it serves as the principal lipid transporter. It has been shown that ApoE directly binds to amyloid β and is needed for its clearance from the brain.[29, 30] There

are three different isoforms of the APOE gene, ε2, ε3 and ε4, which produce three different proteins. Evidence is accumulating that the ε4 variant of the protein has less binding capacity than the ε2 and ε3 variants, and therefore APOE4 carriers are less efficient in clearing amyloid β (Aβ) from the brain.[29] Besides impairment of Aβ clearance, recent studies report that APOE4 carriers also seem to be less efficient in repairing neuronal damage after subarachnoid hemorrhage and traumatic brain injury.[8, 9] So the APOE4 protein is possibly also less efficient in binding other proteins than Aβ. Moreover, there seem to be systemic effects of APOE4 as well. Several studies reported deleterious effects of cardiovascular diseases on cognition and subsequent cognitive decline over time in APOE4 carriers.[31-33] Furthermore, we have found in a previous study that hypertension was associated with higher tau and ptau-181 in APOE4 carriers, but not in non-carriers.[34] More Aβ accumulation in the brain but also less neural plasticity could thus be a consequence of less efficient lipid binding and transport in APOE4 carriers, leading to more oxidative stress and neuronal damage in APOE4 carriers compared to non-carriers.

Longitudinal studies examining CSF F_2-isoprostanes while taking into account both diagnosis and APOE genotype are sparse, and results are conflicting. A study with healthy subjects reported higher F_2-isoprostane levels in APOE4 carriers than in non-carriers.[25] In contrast, a longitudinal study in MCI patients showed no differences in F_2-isoprostane concentration between APOE genotypes.[18] In a cross-sectional study in cognitively normal subjects, CSF F_2-isoprostanes correlated with age in APOE4 carriers but not in APOE4 non-carriers,[35] suggesting presymptomatic oxidative damage in APOE4 carriers. Previous studies assessing longitudinal changes in CSF F_2-isoprostanes have shown an increase over time in AD patients and in MCI patients converting to AD.[22, 23] We have shown earlier that CSF F_2-isoprostanes increased over time in patients from our memory clinic.[24] In the current study we showed that APOE4 carriers had more increase in F_2-isoprostanes over time than APOE4 non-carriers and that it was related to the rate of cognitive decline in APOE4 carriers, while this association was not present in APOE4 non-carriers. This could imply that cognitive decline in APOE4 carriers is partly due to oxidative damage, while in non-carriers only AD pathology or other as yet unraveled pathology is responsible for cognitive decline.

Among the strengths of this study are the large cohort of memory clinic patients with diagnoses throughout the spectrum of cognitively normal to AD and CSF analysis at two time points. In addition, we had the availability of a long clinical follow-up of our patients (3.9±2.4 years). The cognitive decline was therefore measured over an interval nearly twice as long as the change in biomarker concentration (time between LP's was 2.0±1.1 years). This allowed us to relate the increase in F_2-isoprostanes to future cognitive decline. A possible limitation is the fact that inclusion of non-demented patients was biased towards patients who showed decline, as these patients are more likely to return to the clinic and undergo a second lumbar puncture. Fifty percent of the non-demented patients progressed to MCI or

even dementia, and an even larger proportion of MCI patients developed AD at follow up, potentially explaining why there was no difference in F_2-isoprostane concentration between diagnostic groups at baseline, as previously reported.[24] To replicate these findings future studies should address diagnosis and APOE effects in a larger sample, comprising more cognitively stable control subjects. However, the present study was not set up to investigate the diagnostic value of CSF F_2-isoprostanes, but rather to relate F_2-isoprostanes to APOE genotype and cognitive change over time. We had a large number of MMSE scores available with a median of 4 MMSE scores per patient. With these repeated measures we were able to use linear mixed models, which have the advantage that they make use of all available data points, and account for both baseline MMSE and different time intervals.

Our results reiterate the importance of oxidative stress in neurodegeneration, notably in patients carrying the APOE4 gene. Besides, they suggest that F_2-isoprostanes might be valuable, for example in medication trials, as a marker to monitor oxidative stress during treatment in this specific subset of patients. In a phase 2 trial investigating an anti-amyloid-β antibody as treatment for AD potential positive treatment effects were found in APOE4 non-carriers only, while the adverse events (mainly vasogenic edema) were more frequently seen in APOE4 carriers, suggesting even possible harmful instead of beneficial effects of the treatment for APOE4 carriers.[36] With the possibility of increased vulnerability to oxidative damage in APOE4 carriers in mind, it might be important to take the differences between APOE4 carriers and non-carriers into account when performing such trials. How this implicates therapeutic research efforts is subject for further study.

Acknowledgements

Research of the VUmc Alzheimer center is part of the neurodegeneration research program of the Neuroscience Campus Amsterdam. The VUmc Alzheimer center is supported by Alzheimer Nederland and Stichting VUmc fonds. The clinical database structure was developed with funding from Stichting Dioraphte. Stichting De Merel supported the expenses of CSF F_2-isoprostane measurements, and M.I. Kester was supported by a fellowship of Alzheimer Nederland.

Disclosures

P. Scheltens has served as consultant for Wyeth-Elan, Genentech, Danone, and Novartis, and received funding for travel from Pfizer, Elan, Janssen, and Danone Research. C.E. Teunissen is a member of the Innogenetics International Advisory Board. F.H. Duits, W.M. van der Flier, M.I. Kester, P.G. Scheffer and M.A. Blankenstein report no conflicts of interest.

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